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Phenolic Contents and Antioxidant Activities of Various Infused Tea Liquids Made from Leaves of Green Tea (*Camellia sinensis*), Banaba (*Lagestroemia specios*a) and Moringa (*Moringa oleifera* L.)

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ABSTRACT

Plants, such as green tea (*Camellia sinensis*), banaba (*Lagestroemia speciosa*) and moringa (*Moringa oleifera* L.), have been known as the basis of traditional medicine that have been existed for thousands years due to their bioactive compounds. This research aimed to investigate total phenolic content and antioxidant properties of various tea infusion made from green tea, moringa and banaba leaves. Dried green tea, dried banaba leaves, and fresh moringa leaves were used in this study. Fresh moringa leaves then was dried at two different temperatures (50 and 60 ºC). The results showed that the total phenolic content of tea infusions were ranged from 11–20 mg GAE/g, while the DPPH scavenging activity and FRAP activity ranged from 6–12 µmol TE/g, 0.2–0.65 mmol $FeSO4$/g, respectively. Banaba tea had the highest total phenolic content (20.54±0.31 mg GAE/g), DPPH (11.19±0.12 µmol TE/g), and FRAP activity (0.66±0.02 mmol $FeSO4$/g) compared to another infused tea ($P<0.05$). Higher drying temperature (60 ºC) of moringa leaves reduced the total phenolic content in the moringa tea. However, it had no effect to the DPPH scavenging activity and FRAP activity ($P<0.05$). In conclusion, the total phenolic content and antioxidant activities were varied by tea leaf variations and drying condition.

Keywords: Antioxidant, Banaba, Green Tea, Moringa, Polyphenols

INTRODUCTION

Some fresh or dried herbs and leaves have been used for refreshing drinks preparation and medicinal herbal infusions (Pijlac-Zegarac, Samec and Piljac 2013). According to Zhao, Deng, Chen and Li (2013), herbal tea is normally consumed as a beverage brewed from the leaves, fruits, flowers, seeds, stems or roots of plant species and it has been used for thousands of years in many countries for health care and diseases prevention.

The Northern region of Thailand has been known for producing high quality tea. According to Chueamchaitrakun (2014), more than 16,000 hectares have been used for tea cultivation, mostly in Chiang Rai, Chiang Mai, Nan, Lampang, Phrae and Mae Hong Son provinces nowadays. In the last decade, the production of fresh tea leaves in Thailand increased because of expansion of tea planting areas. With the awareness of health benefits and the effort of marketing, demand for tea in Thailand also increased dramatically (Pringpuangkeo, 2014). Green tea is produced from freshly harvested leaves and then steamed immediately to prevent fermentation (Chacko et al., 2010). The steaming process preserves the natural polyphenols in green tea because it destroys the enzymes that can oxidize bioactive compound in green tea which have significant effects on human health (Zhu and Jing, 2013).

Instead of green tea, *Lagerstroemia speciosa*, commonly known as Crepe Myrtle or queen's flower or banaba, is a popular folk medicine in Southeast Asia. A tea from the leaves has been used for the treatment of diabetes mellitus (Judy et al., 2003). The leaves contain large amounts of phenolic compound, such as corosolic acid, which has previously been shown to possess antidiabetic properties and significant amounts of tannins (Hayashi et al., 2002).

In addition, some herbs has been known as a good source of bioactive compounds. *Moringa oleifera* L., commonly called Moringa, is used in traditional medicines due to its various pharmacological properties (Anwar et al., 2007). The major phytochemical constituents in the leaves are phenolic compounds and flavonoids such as cryptochlorogenic acid, isoquercetin, astragalin and some phenolic compound such as chlorogenic acid (Verma et al., 2009, Vongsak et al., 2012, and Mbikay, 2012). These compounds are famous for their wide ranged activities including antioxidation, antihypertension and anti-inflammation (Verma et al., 2009).

Due to the health benefit potential of green tea (*Camellia sinensis*), banaba (*Lagestroemia speciosa*) and Moringa (*Moringa oleifera* L.) leaves, the objective of this study was to determine the total phenolic content and antioxidant activity (DPPH and FRAP assays) of those various infused tea liquids.

METHODOLOGY

Materials

Dried green tea (*Camellia sinensis* var. assamica) leaves (Suwirun®) were obtained from traditional market in Chiang Rai, Thailand. Dried banaba (*Lagestroemia speciosa*) leaves were obtained from Surathani hospital, Thailand. Fresh moringa (*Moringa oleifera* L.) leaves were harvested in Chiang Rai, Thailand.

Dried Leaves Preparation

Leaves were dried with different condition based on the manufacture that produce the leaves commercially. Dried green tea from Suwirun® were prepared by pan-frying young tea leaves variety Assam (*Camelia sinensis* var. assamica). The pan-fried leaves then were rolled, dried and packed into an alumunium bag. In the other hand, dried banaba leaves were prepared from Surathani Hospital by washing the fresh leaves and then sun dried for 1-2 days. After that, dried banaba leaves were packed into a plastic bag. Moringa leaves that harvested freshly were dried in hot air oven at two different temperatures (50 and 60°C) until the final moisture content reach 7%. All the dried leaves then were kept at room temperature for further analysis.

Infused Tea Liquids Preparation

The dried leaves (2 g) were weighed then infused by pouring boiling water (150 ml) at 95±2ºC followed the instruction of commercial tea infusion from Suwirun®. After that, teas were brewed while stirred for 3 min and filtered through Whatman paper filters No. 2. The volume of the infusion was noted. Tea infusions then were analyzed for total phenolic content, DPPH scavenging activity, and ferric reducing antioxidant power activity.

Determination of Total Phenolic Content

Total phenolic content (TPC) was determined according to the method described by the ISO 14502-1 (2005) using gallic acid as a standard. Diluted sample (1.0 ml) was put into a tube. Then, 5.0 ml of 10% v/v Folin-Ciocalteu reagent and 4.0 ml of 7.5% w/v sodium carbonate solution were added with mixing. The mixtures were left for 1 hour at room temperature and the absorbance measured using Genesys 10S UV-Vis Spectrophotometer at 765 nm and using water as blank.

Determination of DPPH Radical Scavenging Activity

The DPPH assay was determined according to the method of Molyneux (2004) using trolox as standard. Diluted sample (50 µl) was mixed with 1.95 ml 60 µM DPPH solution and left in the dark place for 30 min at room temperature. Absorbance measured using Genesys 10S UV-Vis Spectrophotometer at 517 nm and using methanol as blank.

Determination of ferric reducing antioxidant power

Ferric reducing antioxidant power will be assayed by Benzie and Szeto (1999) with few of modification using ferrous sulfate as standard. FRAP reagent were prepared by mixing acetate buffer 300 mM pH 3.6, TPTZ solution 10 mM dissolve in HCl 40 mM, and ferric chloride solution 20 mM with ratio 10:1:1. Diluted sample (400 µl) was mixed with 2.6 ml FRAP reagent. The mixtures then were incubated at 37 ºC for 30 min. Absorbance measured using Genesys 10S UV-Vis Spectrophotometer at 595 nm and using water as blank.

RESULTS AND DISCUSSIONS

Total Phenolic Content of Infused Tea Liquids

Polyphenols are the main compounds in green tea, moringa tea, and banaba tea. Banaba tea contains corosolic acid as the main bioactive compound, while green tea contains catechins and moringa contains cryptochlorogenic acid (Chacko et al., 2010; Vongsak et al., 2012). Banaba tea had the highest TPC (20.54±0.31 mg/g), followed by green tea (14.62±0.44 mg/g), moringa tea that leaves dried at 50 ºC (12.23±0.40 mg/g), and moringa tea that leaves dried at 60 ºC (11.41±0.67 mg/g) ($P<0.05$) as shown in Figure 1.

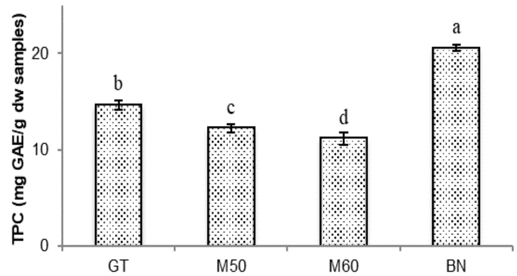

Figure 1. Total phenolic content of infused tea leaves. *GT* green tea, *M50* moringa tea that leaves dried at 50ºC, *M60* moringa tea that leaves dried at 60ºC, *BN* banaba tea. Different letters of superscript on the column indicate significant differences (N = 3).

Figure 1 also shows that TPC of green tea in this study (14.62±0.44 mg/g db) were lower than the values shown in previous studies (24.20±0.3 mg/g db) (Rodrigues et al., 2016). TPC of moringa tea in this study also were lower than phenolic content of moringa leaves grown in Chad (28.13±0.51 mg/g db), Sahrawi (35.52±3.88 mg/g db), and Haiti (25.45±1.94 mg/g db) reported by Leone et al. (2015). In addition, the present results (per g wet basis) were lower than TPC of mature moringa leaves (45.81±0.02 mg/g wb) and young moringa leaves (36.02±0.01 mg/g wb) reported by Sreelatha and Padma (2009). In this study, all the value of TPC of tea infusions is presented in dry basis, however, TPC of banaba tea (per g wet basis) showed lower value compared with Saumya and Mahaboob (2011) (66.83±0.27 mg/g plants). Less TPC of infused teas in this study might be due to different method of extraction that it followed the commercial tea consumption. Infused teas were extracted with less amounts of samples (2 g in 150 ml) using boiling distilled water (not other organic solvents) for shorter time (3 min) and also did not be concentrated compared to other studies. Sreelatha and Padma (2009) extracted the moringa leaves with higher amount of the leaves using soxhlet for longer time (18-20 h) which infused with water and concentrated under controlled temperature (40–50ºC).

Drying can cause changes in the food properties including discolouring, aroma loss, textural changes, and nutritive value (Madrau et al., 2009). Different drying temperature (50 and 60ºC) affected the TPC of moringa leaves infusion (Figure 1). Higher temperature of drying process can destroy more phenolic content in moringa leaves (Leone et al., 2015) caused decreasing TPC of moringa tea significantly (P<0.05). Mudau and Ngezimana (2014) also reported that different drying temperature (60, 100, and 140ºC) significantly affected phytochemical compositions of bush tea. Higher drying temperature reduces the drying time but resulted in less product quality, heat damage to the surface and higher energy consumption. However, mild drying conditions with lower temperature improved the product quality but decrease the drying rate thus drying period is lengthened (Kumar et al., 2014).

Antioxidant Properties of Infused Tea Liquids

DPPH scavenging activity of tea infusion expressed as mmol trolox equivalent per gram dry weight samples as shown in Figure 2. Banaba tea had the significant highest DPPH scavenging activity (11.19±1.20 μmol TE/g DB) among the raw materials infusion followed by green tea (10.30 ±4.62 μmol TE/g DB) and moringa tea that leaves dried at 50 (6.08±8.38 μmol TE/g DB) and 60ºC (6.11±6.43 μmol TE/g DB) (P<0.05). It was slightly different from previous study (Anil et al., 2010; Anissi et al., 2014; Leone et al., 2015). It would be due to difference of sources of raw material and drying temperature. Temperature higher than 75ºC could be destroy more phenolic (Suvarnakuta et al., 2014) that related to antioxidant activity.

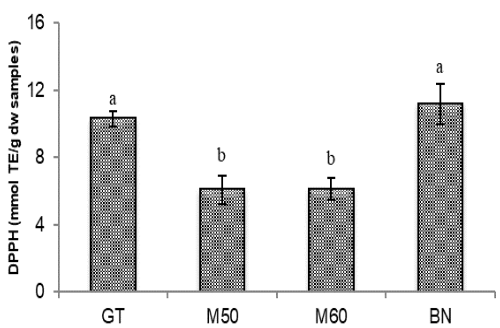

Figure 2. DPPH scavenging activity of infused tea leaves. *GT* green tea, *M50* moringa tea that leaves dried at 50ºC, *M60* moringa tea that leaves dried at 60ºC, *BN* banaba tea. Different letters of superscript on the column indicate significant differences (N = 3).

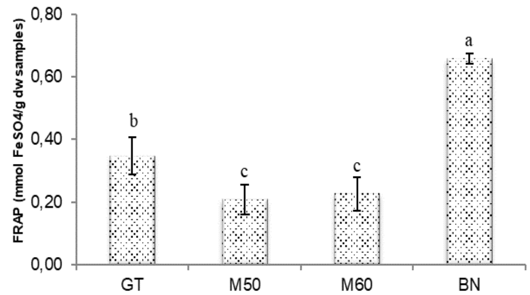

Figure 3. Ferric reducing antioxidant power of infused tea leaves. *GT* green tea, *M50* moringa tea that leaves dried at 50ºC, *M60* moringa tea that leaves dried at 60ºC, *BN* banaba tea. Different letters of superscript on the column indicate significant differences (N = 3).

The antioxidant activity of phenol was mainly due to their redox properties, hydrogen donors and singlet oxygen quenchers (Sreelatha and Padma, 2009). On interaction with DPPH, antioxidants neutralize its free radical character by either transferring an electron or hydrogen atom (Naik et al., 2003). The degree of discoloration of DPPH indicates the scavenging potential of the antioxidant extract, which is due to the radical scavenging ability (Sreelatha and Padma, 2009). Polyphenols were one of the main antioxidants in infused tea. Therefore, the more TPC in tea liquid samples, the more antioxidant activities were found. There was highly positive correlation (R^2 = 0.988) between antioxidant activity and TPC. Previous studies also reported the same correlation (R^2 = 0.9551) (Chen et al., 2015).

Figure 3 shows ferric reducing activity power power (FRAP) of infused tea liquids. FRAP of banaba tea (0.65±0.01 mmol/g) was considerably higher than green tea (0.35±0.06 mmol/g), moringa tea that leaves dried at 50ºC (0.21±0.05 mmol/g), and moringa tea that leaves dried at 60ºC (0.23±0.05 mmol/g) (P<0.05). The results indicated similar trend with DPPH scavenging activity and TPC. It is well known that phenolic acids are highly effective free radical scavengers and metal chelator (Wan et al., 2008). It was found that ferric reducing activity power and TPC had high positive correlation (R^2 = 0.979) similar with previous study (R^2 = 0.9458) (Chen et al., 2015). Therefore, the high amount of phenolic content affected to the antioxidant activity to the tea samples.

The drying temperature at 50 and 60ºC had no influences on the DPPH scavenging activity and ferric reducing power activity power of moringa leaves (P<0.05) as shown in Figures 2 and 3. According to Suvarnakuta et al. (2014), 75ºC was proposed as an appropriate drying technique and condition to preserve polyphenol in mangosteen pericarp. Therefore, drying at 60ºC still could prevent the damage of polyphenol compounds. Larrauri et at. (1997) also reported that drying at 60ºC did not significantly affect the TPC and antioxidant activity in red grape pomace peels.

CONCLUSION

Infused tea made from banaba leaves had the highest total phenolic content, DPPH scavenging activity, and FRAP activity. Moreover, drying temperature at 50 and 60ºC had significantly affected the total phenolic content but it had not affected the antioxidant activities of infused moringa tea liquid. The dried leaves, such as green tea, banaba, and moringa, that had been used for this study were prepared from different drying conditions. Therefore, it will be better to use the fresh leaves and dry in the same drying condition for further research determination.

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